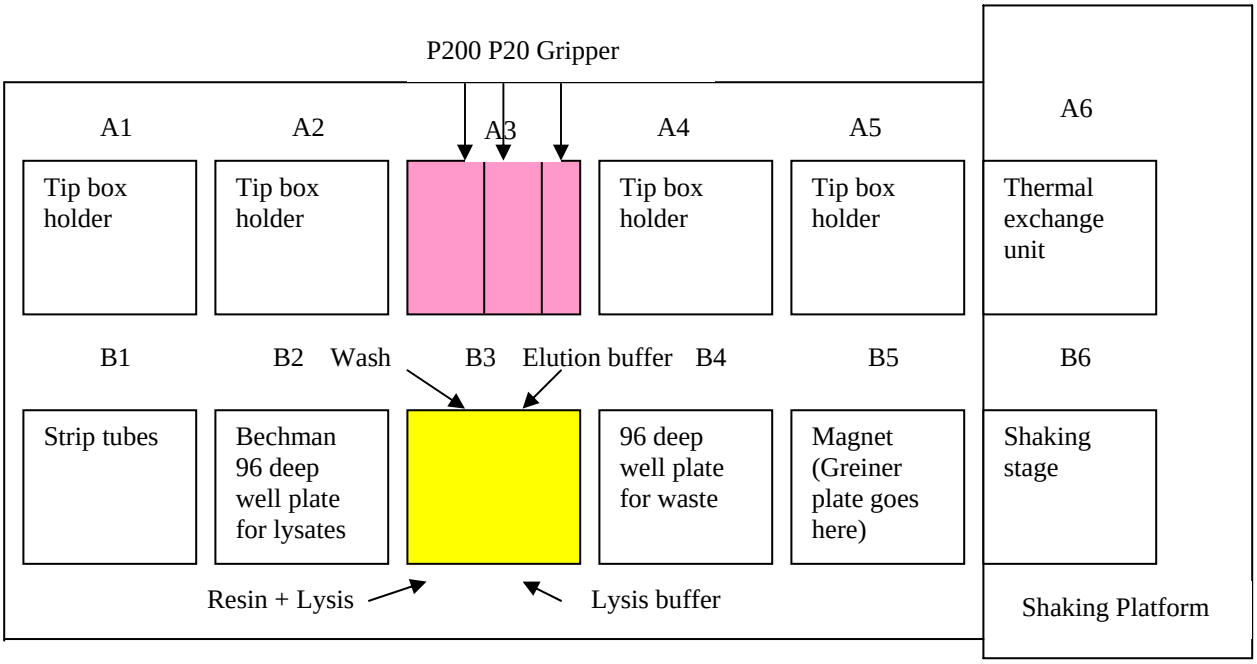
2 PREPARATION OF THE BIOMEK®2000 AUTOMATION WORKSTATION FOR DNA ISOLATION

2.1 TECHNICAL NOTES

2.1.1 Unless specifically indicated, no substitution for the plates or other consumables can be made. Do not substitute another brand because small differences in the well formation or depth of the well can cause the tips to "bottom out". When tips "bottom out" the tips are in contact with the bottom of the well which can form a vacuum in some tips when the tool aspirates liquid and affect the accuracy of pipetting.

2.1.2 Detailed view of the BioMek® 2000 Workstation deck:



2.1.3 The water bath connected to the BioMek® 2000 Workstation set at a temperature of 75°C is used to heat the thermal exchange unit for eluting the DNA from the magnetic resin.

2.1.4 The DNA IQ™ Lysis buffer is a proprietary detergent mixture containing Guanidine Thiocyanate (GTC). The GTC is a chaotropic agent, necessary for the DNA to stick to the silica-coated, paramagnetic resin.

2.1.5 The DNA sticks to the resin through hydrophobic interactions, however the exact mechanism is unknown.

2.1.6 The DNA IQ™ Wash Buffer is a low salt buffer, which is 50% alcohol (50% isopropyl: 50% ethanol), and 50 % NaAcetate. The alcohol in the wash keeps the DNA stuck to the resin and the low salt buffer helps to remove excess salt (Guanidine Thiocyanate) from the DNA stuck to the resin. If the Guanidine Thiocyanate is not removed this could interfere with the PCR amplification. .

2.1.7 The DNA IQ™ Elution Buffer is added in order to release the DNA from the resin. If the temperature at which the material is heated (i.e., 56ºC) is not sufficient, the yield of DNA obtained will be lower than expected.

2.1.8 Plate blanks are utilized during the BioMek 2000 Automation Workstation/DNA IQ™ Isolation process to monitor the system for carry-over contamination that may occur between columns and between rows. The plate blank is carried through the entire process to the typing gel phase.

2.1.9 When the DNA is initially eluted from the DNA IQ™ paramagnetic resin some of it is single stranded and therefore cannot be quantitated on an agarose gel.

2.2 EQUIPMENT

2.2.1 BioMek®2000 Automation Workstation

2.2.2 Magna bot

2.2.3 Heating circulator

2.2.4 Water bath

2.2.5 Heat transfer block

2.2.6 Computer with BioWorks Software

2.2.7 Shaker

2.2.8 Pipettes – 100 µL, 200 µL, and 1000 µL

2.2.9 96 well thermal cycler rack

2.2.10 4-Beckman 24 Microfuge tube holders – Beckman Catalog # 373661

2.2.11 Beckman white 1.5 mL tube inserts – Beckman Catalog # 373656

2.3 MATERIALS

2.3.1 Greiner U-bottom 96 well plates (Do not substitute these plates with a different brand)

2.3.2 Beckman 96 deep well plates (Do not substitute these plates with a different brand)

2.3.3 8-Reaction tube strips, 0.2 mL

2.3.4 8-Cap strips, 0.2 mL

2.3.5 Beckman P20 tips

2.3.6 Beckman P250 tips

2.3.7 Quarter module reservoirs

2.3.8 Tape

2.3.9 Sterile ART tips for pipettes - 100 µL, 200 µL, and 1000 µL

2.4 REAGENTS

2.4.1 DNA IQ™ System Reaction Kit

2.4.2 DNA IQ™ Lysis Buffer

2.4.3 DNA IQ™ Wash Buffer

2.4.4 DNA IQ™ Resin

2.4.5 DTT

2.4.6 DNA IQ™ Elution Buffer

2.5 STARTING THE BIOMEK® 2000 AUTOMATION WORKSTATION

2.5.1 Turn on the computer.

2.5.2 Turn on the BioMek® 2000 Automation Workstation using the power button located on the back left side of the unit under the power cord.

2.5.3 Turn on the Shaker by using the power button located on the back of the unit.

2.5.4 Turn on the water bath and ensure the water level is within 1-2 inches from the top. If more water needed, add deionized tap water to the bath.

2.5.5 Complete the BioMek® 2000 Automation Workstation/AluQuant® Human Quantitation System loading sheet (Appendix D, form BM-2 or the electronic Excel version) to reflect in which well each sample will be loaded.

2.5.6 Place the pre-labeled and capped 1.5 mL microcentrifuge tubes into the four Beckman 24 Microfuge tube holders on the work bench. The pre-labeled tubes will be placed into the Microfuge tube holders in the same order as the samples listed on the 96 deep well plate loading sheet. These tubes will be set aside and used after the DNA Normalization Wizard procedure when the extracted DNA is prepared for permanent storage.

2.5.6.1 Place the pre-labeled attached cap PCR amplification tubes into a 96 well black PCR support base. The pre-labeled tubes will be placed into the support base in the same order as the samples listed on the 96 well plate loading sheet, except the tubes will be spaced into the support bases such that an empty column will be left between each column of tubes to accommodate the attached caps. Therefore the tubes will be spaced over two 96 well PCR support bases. Refer to the figures 19A and 19B in Chapter 5 as a reference for the tube setup. The attached cap should be in a 2 O'clock position.

NOTE: If knowns and unknowns are loaded into the same 96 deep well plate the knowns and unknowns must be separated by a column of reagent blanks.

2.5.7 Depending on the sample type pipette the following volume of sample into the appropriate well of a clean 96 deep well plate. If it is suspected that a high concentration of DNA may be present, it is acceptable to load proportionally less volume than what is specified below:

2.5.7.1 For buccal cell type samples and bloodstains, pipette up to 275 uL of the lysate.

2.5.7.2 For non-sperm fractions, pipette up to 100 uL of the lysate.

2.5.7.3 For sperm fractions, pipette entire sample (this is approximately 30-50 uL of the sperm cell suspension). Take care to transfer the entire sperm pellet.

2.5.7.4 Hair samples (optional: envelopes, stamps, cigarette butts, flakes of blood, and other low level samples) - 100 µL of the lysate.

2.5.8 Loading evidence samples:

2.5.8.1 Once a column of evidence samples has been loaded into the 96 deep well plate, place a piece of tape over the wells to prevent other samples from being pipetted into the same well.

Plate with Knowns and Unknowns

A1	A2	A3 Blank	A4	A5 Blank	A6	A7	A8 Blank	A9 Known	A10	A11	A12
B1	B2	B3 Blank	B4	B5 Blank	B6	B7	B8 Blank	B9 Known	B10	B11	B12
C1	C2	C3 Blank	C4	C5 Blank	C6	C7	C8 Blank	C9 Known	C10	C11	C12
D1	D2	D3 Blank	D4	D5 Blank	D6	D7	D8 Blank	D9 Known	D10	D11	D12
E1	E2 Blank	E3 Blank	E4	E5 Blank	E6	E7	E8 Blank	E9 Known	E10	E11	E12
F1	F2 Blank	F3	F4	F5 Blank	F6	F7	F8 Blank	F9 Known	F10	F11	F12
G1	G2 Blank	G3	G4	G5 Blank	G6	G7	G8 Blank	G9 Known	G10	G11	G12
H1	H2 Blank	H3	H4	H5 Blank	H6	H7	H8 Blank	H9 Known	H10	H11	H12

Figure 1. Plate with knowns and unknowns.

2.5.8.2 If an examiner has already loaded evidence samples into the 96 deep well plate (i.e., Figure 1, wells A1 through D2) the next examiner will load his/her evidence samples in a format that will prevent the evidence samples from one examiner being next to another examiner's samples (i.e., F3 through H4 and A6 through H7). Refer to Section 3, Quality Assurance Measures For The BioMek® 2000 Automation Workstation, for information on plate controls.

2.5.8.3 Knowns and unknowns may be loaded on the same 96 well plate or on separate plates as represented in Figures 2 and 3 below.

Plate with Only Unknowns

A1	A2	A3 Blank	A4	A5 Blank	A6	A7	A8 Blank	A9 Blank	A10 Blank	A11 Blank	A12
B1	B2	B3 Blank	B4	B5 Blank	B6	B7	B8 Blank	B9 Blank	B10 Blank	B11 Blank	B12
C1	C2	C3 Blank	C4	C5 Blank	C6	C7	C8 Blank	C9 Blank	C10 Blank	C11 Blank	C12
D1	D2	D3 Blank	D4	D5 Blank	D6	D7	D8 Blank	D9 Blank	D10 Blank	D11 Blank	D12
E1	E2 Blank	E3 Blank	E4	E5 Blank	E6	E7	E8 Blank	E9 Blank	E10 Blank	E11 Blank	E12
F1	F2 Blank	F3	F4	F5 Blank	F6	F7	F8 Blank	F9 Blank	F10 Blank	F11 Blank	F12
G1	G2 Blank	G3	G4	G5 Blank	G6	G7	G8 Blank	G9 Blank	G10 Blank	G11 Blank	G12
H1	H2 Blank	H3	H4	H5 Blank	H6	H7	H8 Blank	H9 Blank	H10 Blank	H11 Blank	H12

Figure 2. Plate with only unknowns

Plate with Only Knowns

A1 Known	A2 Blank	A3 Blank	A4 Blank	A5 Blank	A6 Blank	A7 Blank	A8 Blank	A9 Blank	A10 Blank	A11 Blank	A12
B1 Known	B2 Blank	B3 Blank	B4 Blank	B5 Blank	B6 Blank	B7 Blank	B8 Blank	B9 Blank	B10 Blank	B11 Blank	B12
C1 Known	C2 Blank	C3 Blank	C4 Blank	C5 Blank	C6 Blank	C7 Blank	C8 Blank	C9 Blank	C10 Blank	C11 Blank	C12
D1 Known	D2 Blank	D3 Blank	D4 Blank	D5 Blank	D6 Blank	D7 Blank	D8 Blank	D9 Blank	D10 Blank	D11 Blank	D12
E1 Known	E2 Blank	E3 Blank	E4 Blank	E5 Blank	E6 Blank	E7 Blank	E8 Blank	E9 Blank	E10 Blank	E11 Blank	E12
F1 Known	F2 Blank	F3 Blank	F4 Blank	F5 Blank	F6 Blank	F7 Blank	F8 Blank	F9 Blank	F10 Blank	F11 Blank	F12
G1 Known	G2 Blank	G3 Blank	G4 Blank	G5 Blank	G6 Blank	G7 Blank	G8 Blank	G9 Blank	G10 Blank	G11 Blank	G12
H1 Known	H2 Blank	H3 Blank	H4 Blank	H5 Blank	H6 Blank	H7 Blank	H8 Blank	H9 Blank	H10 Blank	H11 Blank	H12

Figure 3. Plate with only knowns

2.5.9 Loading known/reference samples:

2.5.9.1 If known/reference samples are loaded into the sample 96 deep well plate a column of plate blanks will be used to separate the known/reference samples from the evidence samples as demonstrated in the first diagram above (i.e., wells A8 through H8).

2.5.9.2 Known/reference samples will be loaded sequentially (i.e., A9 – H9). It is not necessary to have known/reference samples from one examiner separated by an empty well from another examiner's known/reference samples. A columns of reference samples may be loaded adjacent to another columns of reference samples. Refer to Section 3, Quality Assurance Measures for The BioMek® 2000 Automation Workstation, for information on plate controls.

2.5.10 Proceed to Section 2.6, BioMek® 2000 Automation Workstation Operating Procedure.

2.6 BIOMEK® 2000 AUTOMATION WORKSTATION OPERATING PROCEDURE

An EMERGENCY STOP BUTTON is located at the lower front area on the robot, just below the deck. If this button is pressed, the robot will abort the method it is currently running. The emergency stop only needs to be used when the robot could be damaged by crashing into the deck or a tool could be damaged by crashing into something on the deck. If what is desired is simply to stop the method while it is running, simply click on the "Stop" button. The method will then pause and several options will be available. The button labeled "Continue" can be used to resume the method, the button labeled "Trace" can be used to advance to the next step (this function is useful when calibrating), the button labeled "Go Up" will move the pod up and the button labeled "Quit" will terminate the method.

When the BioWorks robot program is initiated it will prompt the user regarding the proper placement, number and type of pipette tips to use (either Beckman P20 or P250 tips). A prompt will also ask about the temperature of the water bath. Each method has prompts that will ask if the correct volume of reagent has been placed into the modular reservoir at the appropriate position. A series of prompts will come up when a method is initiated asking whether or not the water bath is at temperature, the tip boxes are appropriately placed and if the proper volume of the various reagents has been placed into the modular reservoir at the appropriate position. Responding to the prompts will ensure the user has correctly set up the deck for the selected method. **All reagents used for the DNA IQ™ ISOLATION SYSTEM must be properly prepared prior to use.**

NOTE: Some of the reagents are sensitive to evaporation, **DO NOT** fill the reservoirs for the BioMek® method until just prior to initiating the robot run.

2.6.1 Methods for running specific programs on the BioMek® 2000 Automation Workstation are located in the BioWorks folder. Click on the BioWorks folder located on the desktop.

2.6.2 The open folder contains a number of software program icons. To select a method, double click on the Edit icon. Then, either using the method drag-down window or using the open folder icon, select Open. The following window, Figure 4, will open and a list of all methods will be displayed (i.e., 40 sample method). Double click on the method you wish to open or highlight the method and click on the Open button.

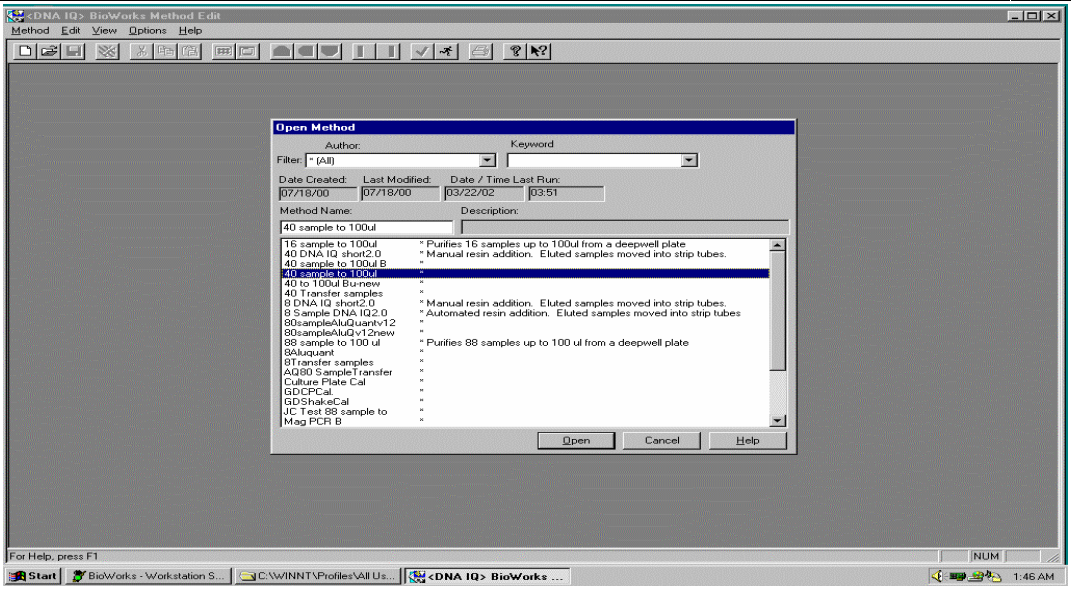


Figure 4. List of available methods

NOTE: Before utilizing the BioMek® 2000 Automation Workstation for DNA extraction, a test of the shaker MUST be performed. This needs to be performed once a day when starting up the computer, BioMek® 2000 Automation Workstation, and shaker, unless there is reason to believe that the shaker has come "off-line" with the workstation and computer. A method, the Shaker Test, was created to test whether or not the shaker is "on line" with the workstation and computer. When trying to execute the Shaker Test method, if an error message box comes up stating that the computer is unable to communicate with the shaker, the entire system needs to be shut down and re-started in the correct sequence (computer, workstation, and shaker). The shaker test must then be repeated. The Shaker Test method doesn't need to run to completion. Once the robot has precisely picked up a Greiner plate from the magnet, moved it to the clamp on the shaker platform and initiated shaking according to the computer command without any error messages, the method can be aborted.

2.6.3 Shaker Test (run once a day when starting up the computer, BioMek® 2000 Automation Workstation, and shaker):

2.6.3.1 Using the Edit function, open up the Open Method window.

2.6.3.2 Scroll to the bottom of the window where the Shaker Test method is visible. Double click on the Shaker Test or highlight it then click "Open". The method will open up with an inset window showing the deck layout. Disregard the deck layout other than having the appropriate tools at deck position A3 (most importantly the Gripper tool) and having a Greiner plate on the magnet, at deck position B5.

2.6.3.3 Click on the running man icon on the tool bar to initiate the method. A window will appear showing a stop light with a red light. It will state that the light will turn green once communication has been established. It will take about a minute or less for the computer to establish communication with the robot. Once this occurs, the light will

turn green and the window will disappear. An inset window method will ask if you accept the deck layout, click accept, then the method will initiate.

2.6.3.4 Once the shaker has responded to the computer commands by shaking, the method can be aborted. To do this, click the "stop" button on the inset window that shows the shaker function. The shaker will stop shaking, but the timer will still be running for that function. Close that window and click the "stop" button for the method which is located at the far left of the window. Then click "quit" and the method will completely terminate. Now the robot is ready for executing a DNA extraction method.

2.6.3.5 Double click on the DNA IQ™ extraction method to run the appropriate program depending on the number of samples

NOTE: The prompts and messages for the method will appear in green.

2.6.4 Following the directions provided by the program in green type set up the deck components (i.e., tips, plates, quarter module reservoirs, and buffers). Descriptions of all the reservoirs and their contents are in green type. The deck layout will also come up as represented in Figure 4. This will allow the user to set up all the deck components appropriately for the selected method prior to its execution.

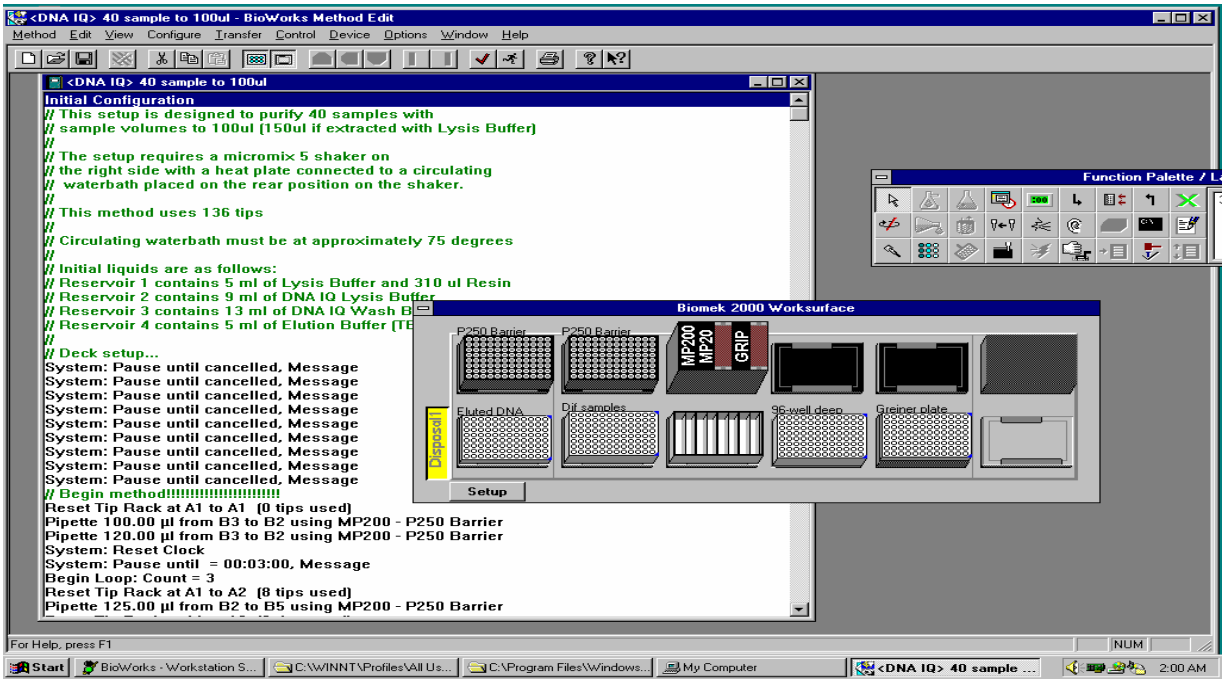


Figure 5. Deck layout for sample method

NOTE: Unless specifically indicated, no substitution for the plates can be made. For example, the 24, 40, 56 and 88 sample methods specifically call for a Beckman brand 96 deep well plate. Do not substitute another brand (such as the Marsh brand) because small differences in the well formation or depth of the well can cause the tips to "bottom out". When tips "bottom out" the tips are in

contact with the bottom of the well and when the tool goes to aspirate liquid, a vacuum can form in some tips, which could affect the accuracy of pipetting.

2.6.4.1 Place a clean, unused Greiner (shallow 96 well plate) onto the magnet located at deck position B5.

2.6.4.2 Place a clean, unused 96 deep well plate for waste at deck position B4

2.6.4.3 Place the appropriate number and type of clean tip boxes in the designated position in row A. Make sure that the tip boxes are fitting squarely into the black clamps. It is necessary to push back on the spring manually to get the tip box into the holder.

2.6.4.4 Place the quarter module reservoirs in the holders at position B3 of the deck.

2.6.4.5 In deck position B1 place a black 96 well PCR support base. Starting from left to right place the appropriate number of strip tubes depending on the number of samples that are being extracted (i.e., for the 40 sample method, 5 columns consisting of 8–strip tubes each. **ENSURE THAT THE TUBES ARE FULLY SEATED IN THE 96 PCR SUPPORT BASE.**

NOTE: **DO NOT** pre-mix the DNA IQ™ Lysis Buffer containing DTT with the resin. The BioMek® 2000 Automation Workstation will mix these two solutions.

2.6.4.6 Once all samples have been loaded into the plate, place the 96 deep well plate onto the BioMek® 2000 Automation Workstation deck at position B2. Ensure that all tape has been removed from the wells

2.6.4.7 Click on the button showing an image of a running man on the menu bar. This will initiate the method.

2.6.4.8 Once a method has been initiated the following window, Figure 6, will open showing the deck layout appropriate for the selected method. Click on Accept All.

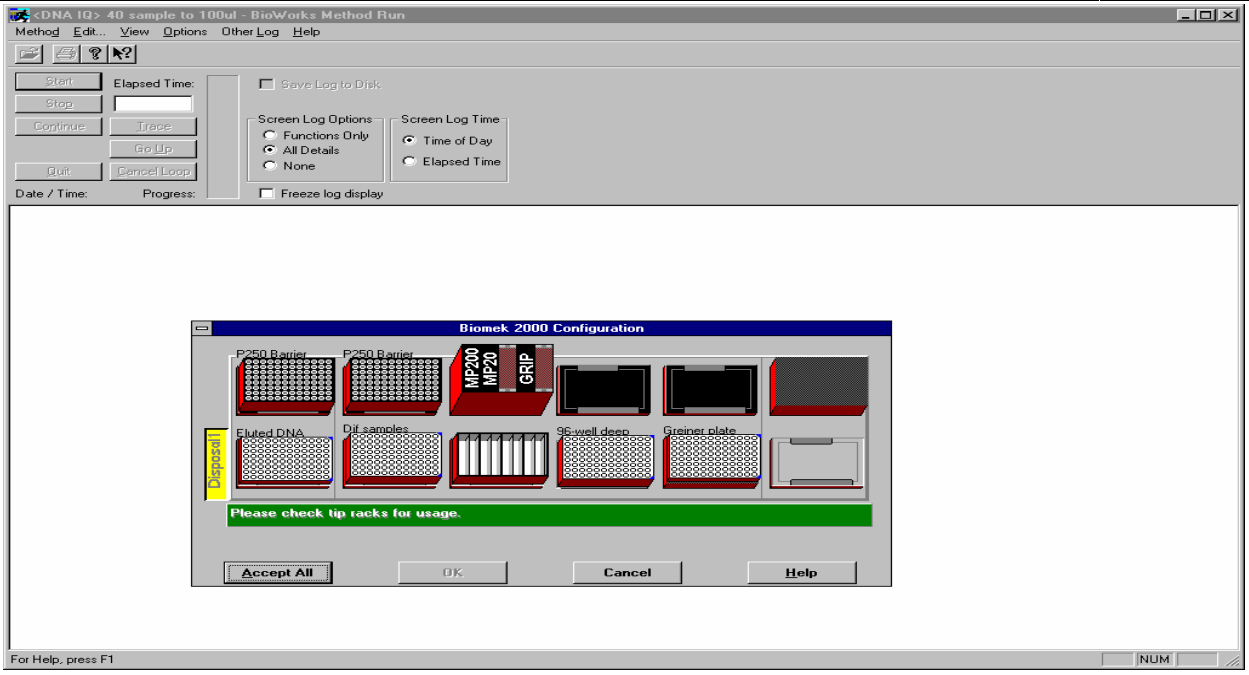


Figure 6. Deck layout for the selected method

2.6.4.9 The robot will initiate the method and will take the user through a series of prompts beginning with a prompt asking whether the water bath is at 75°C. If the water bath is turned on, click OK even if it is not yet up to temperature, it will reach the appropriate temperature by the time the thermal exchange unit is needed. One exception should be noted. The water bath should be close to the appropriate temperature when the 24 sample method is initiated. Because this method is short the water bath will not reach 75°C prior to completing the method if the water bath is turned on just when the method is initiated.

2.6.4.10 The following prompts will ask about the tip boxes, the 96 deep well plates, the Greiner plate and the reagents in the reservoirs. Click on OK for each prompt after verifying that the step has been completed. Once all the steps/prompts have been completed, the robot will start the DNA extraction procedure.

NOTE: Once the DNA extraction is finished, the DNA will be eluted into 40 µL of DNA IQ™ Elution buffer, then pipetted into the strip tubes at deck position B1.

2.6.5 The BioMek® 2000 Automation Workstation will run for approximately 1 hour for the 24 sample method, 1 hour and 15 minutes for the 40 sample method, 1 hour and 45 minutes for the 56 sample method and 2 hours for the 88 sample method.

2.6.6 After the DNA isolation is complete, carry the samples forward for quantitation as outlined in Chapter 4, AluQuant® Human Quantitation System.

♦END